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NITRATE ASSIMILATION BY SOIL BACTERIA

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It has long been known that straw or strawy manure when added to the soil may bring about a reduction in the nitrate content of the soil due to a stimulation of certain biological processes. The conclusion has been drawn that the nitrate may disappear as a result of denitrification, nitrate reduction or nitrate assimilation. In experiments along this line a number of investigators (1, 2, 4, 5) have found that the total nitrogen content was unchanged or only very slightly decreased while large amounts of nitrate nitrogen disappeared. The conditions in the experiments precluded the possibility of denitrification or nitrate reduction and it was concluded, therefore, that the nitrate nitrogen was assimilated by the microorganisms.

Nitrate assimilation has come to be recognized as a distinct and rather common biological process in soils and the frequent occurrence of the process to an undesirable extent emphasizes the need for a study of methods of control and especially of the organisms involved.

The results secured in a study of some nitrate assimilating organisms isolated from soils in which the nitrates had recently disappeared are presented in this paper.

EXPERIMENTAL

Samples of the soil were taken aseptically and enrichment cultures made by using about 10 grams of the moist soil in 100 c.c. of a nitrate solution in 500 c.c. Erlenmeyer flasks. The nitrate solution contained 1 per cent glucose, 0.1 per cent potassium nitrate, 0.05 per cent potassium hydrogen phosphate and 0.02 per cent calcium chloride. The reaction of the solution was adjusted to a pH of 7.0 by the addition of 1 per cent sodium hydroxide. The enrichment cultures were incubated at 25° C. and dilution plates poured on nitrate agar after 3 days. Typical colonies were selected and dilutions made again to secure the organisms in pure culture, after which they were transferred to agar slants of the same medium.

[1] The writers take pleasure in acknowledging indebtedness to Dr. P. E. Brown for his suggestions in connection with this work.

Qualitative tests for nitrate reduction were made according to the methods recommended in the manual of methods for pure culture study of the bacteria. The results secured are presented in table I.

The results show that organisms A and E reduced nitrate to nitrite and ultilized the nitrite as a nitrogen source. No ammonia was detected in the cultures. Organism X did not reduce nitrate to nitrite but some ammonia was formed. This organism did not utilize nitrite as a source of nitrogen. Organism Y reduced nitrate to nitrite but did not utilize the nitrite nor was the presence of any ammonia noted. Organism 5 reduced nitrate to nitrite and did not utilize the nitrite but some ammonia was produced.

The disappearance of nitrate and the changes in total nitrogen content were studied in solution cultures. One hundred c.c. of a nitrate solution similar to that described above were inoculated in 500 c.c. Erlenmeyer flasks with a 1 c.c. suspension of the organism. Quadruplicate flasks were inoculated with each organism. Dilution plates were poured in the original inoculum and after 7 days' incubation, nitrate nitrogen was determined on the original medium and after incubation by the Devarda reduction method. Total nitrogen was determined in the original medium and in the inoculated solutions after incubation by the Davisson and Parsons method as recommended by Fred and Waksman (3). The results secured are presented in table 2.

Good growth was secured in cultures A. E. and X but cultures Y and especially 5 were poor. Where there was growth there was almost a complete disappearance of the nitrates and the total nitrogen content was practically unchanged. In the solution inoculated with organism A there was a loss of 1.26 mgms. of total nitrogen, whereas in the solution inoculated with organism X there was an increase of 0.07 milligram of total nitrogen. However, these amounts are small enough to fall within experimental error, and are not considered significant, especially since more than 11.00 milligrams of nitrate nitrogen disappeared and the amount of ammonia found was small. Organisms Y and 5 did not utilize nitrite and only small amounts of nitrate disappeared in solutions inoculated with these organisms. However, since growth was poor, these results are not significant.

Conclusions

It is concluded from these results that certain soil bacteria may assimilate large quantities of nitrate nitrogen under favorable conditions of moisture, temperature and food supply.

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Table I — Nitrate Reduction

Culture	NO_2 in NO_3 medium	NO_2 in NO_2 medium	NH_3 in NO_3 medium
Control	000	+++	000
A	+++	000	000
E	+++	000	000
X	000	+++	+++
Y	+++	+++	000
5	+++	+++	+++

Table II — Nitrate Assimilation in Solution Cultures

Culture	No. of Organisms		Mgm. NO_3—N			Mgm.	Mgm. NH_3—N.
	Original Seeding	After 7 Days	In Beginning	After 7 Days	Assimilated in 7 Days	Total N after 7 Days	
Control	0	0	12.26	12.26	—	13.87	0.39
A	430,750	21,930,000	12.26	0.98	11.28	12.61	0.42
E	1,425,000	*	12.26	0.97	11.29	13.59	0.28
X	784,000	*	12.26	0.93	11.33	13.94	0.35
Y	*	12,250,000	12.26	11.14	1.12	—	0.35
5	2,800	0	12.26	11.25	1.01	—	0.39

* Too many colonies to count.